High Production Volume (HPV) Challenge Program

Test Plan

For

AMPS® Category

Prepared by

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1.0 INTRODUCTION

On March 2, 1999, the Lubrizol Corporation committed to provide basic toxicity information on chemicals listed under the Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program. The sponsored chemicals addressed in this test plan are:

2-acrylamido-2-methylpropanesulfonic acid (CASRN 15214-89-8), and

2-acrylamido-2-methylpropanesulfonic acid, sodium salt (CASRN 5165-97-9).

By participating in this voluntary program, The Lubrizol Corporation agreed to assess the adequacy of existing data; design and submit test plans to fill data gaps; provide test results as they are generated; and prepare summaries of the data characterizing each chemical.

The HPV Challenge Program encourages the development of chemical categories as a mechanism to achieve an efficient completion of the program goals. Based upon the guidance issued by the EPA for the composition of a chemical category, and the inherent structural similarities of the HPV chemicals addressed in this test plan, it was determined that 2-acrylamido-2-methylpropanesulfonic acid and 2-acrylamido-2-methylpropanesulfonic acid, sodium salt are ideal candidates for review under a single chemical category. For the purposes of this test plan and the accompanying robust summaries, the chemical group will be referred to as the AMPS® category. In support of the AMPS® category approach, modeling and test data for 2-acrylamido-2-methylpropanesulfonic acid, ammonium salt (CASRN 58374-69-9; a non-HPV chemical) are included in the test plan and robust summaries.

To ease the task of communicating information on the members of the AMPS® category in this test plan, the following abbreviated chemical names will be used:

AMPS® acid (2-acrylamido-2-methylpropanesulfonic acid),

Sodium AMPS® (2-acrylamido-2-methylpropanesulfonic acid, sodium salt);

Ammonium AMPS® (2-acrylamido-2-methylpropanesulfonic acid, ammonium salt)

2.0 EVALUATION OF DATA

The EPA guidance on chemical categories states that structural similarities among members of a category create a predictable pattern in any or all of the following parameters: physicochemical properties, environmental fate and environmental effects, and human health effects. As will be evident from the information provided below, the data findings for the members of the AMPS® category fulfill these criteria and support the inclusion of the acid and its neutral salts as a single chemical family. Consequently, it is valid that data from one or more AMPS® derivatives is used to satisfy a particular environmental, aquatic or health-related endpoint.

The process of evaluating the basic toxicity of the members of the AMPS® chemical category entailed the following stepwise process: grouping of chemicals into a putative category; gathering relevant data for each member of the category; evaluating the compiled data for adequacy; evaluation of the physico-chemical, environmental, aquatic and health-effects data to confirm the correlation between category members; construction of a matrix of SIDS endpoints for the category members; and identification of data gaps for critical endpoints within the AMPS® category (Table 7; illustration of the matrix).

2.1 Physical Chemical Description of the AMPS® Category

A chemical category is defined by EPA for the purposes of the HPV Chemical Challenge Program as a group of chemicals whose physico-chemical and toxicological properties are likely to be similar, or to follow a regular pattern as a result of structural similarity. The members of the AMPS® category are virtually homologous, characterized by a 2-acrylamido-2-methylpropanesulfonic parent anion, distinct only by the corresponding H^+, Na^+ or NH_4^+ counterion (Figure 1).

AMPS® acid is prepared by reacting acrylonitrile, isobutylene, and oleum in the presence of water. The sodium AMPS® and ammonium AMPS® salts are subsequently formed by neutralization of AMPS® acid with sodium hydroxide or ammonium hydroxide, respectively. The AMPS® monomer (parent structure) is a propanesulfonic acid substituted at the C2 position with a methyl group and an acrylamido moiety. Reactive sites on the molecule include the unsaturated vinyl group and the terminal sulfonic acid.

The molecular weight of AMPS® acid is 207, whereas the molecular weight of sodium AMPS® and ammonium AMPS® are 229.2 and 224.2, respectively. The melting point for ammonium AMPS® was estimated using the capillary tube method to be 191°C. The melting point for the AMPS® acid and sodium AMPS® was estimated to be 160.78ºC and 260.35ºC, respectively using the EPIWIN[1] model (EPA and Syracuse Research Corporation). The AMPS® monomer decomposes at, or slightly above, it's melting point temperature. As a result, boiling point determinations are not applicable. Vapor pressure estimations were performed using analytical techniques and mathematical modeling. Gravimetric testing methods estimated the vapor pressure of ammonium AMPS® to be 7.4×10^{-9} Pa @ 25°C. Mathematical modeling using the EPIWIN estimation software estimated the vapor pressure of AMPS® acid and sodium AMPS® to be 6.75×10^{-9} and 1.72×10^{-13} mm Hg at 25ºC respectively. Due to the low vapor pressure, the members of this category will not significantly volatilize into the vapor phase. The octanol-water partition coefficient ($\log_{10}P_{ow}$) for ammonium AMPS® was experimentally determined to be -3.41 @ 22°C. Partition coefficient for the AMPS® acid and sodium AMPS® was estimated to be -2.19 and –4.34 respectively, using the EPIWIN estimation program. AMPS® monomers are hygroscopic and hydrophilic. The water solubility of the ammonium AMPS® acid was experimentally determined to be 76 gm/100 gm water whereas the EPIWIN model projected the water solubility of AMPS®

[1] EPIWIN. Estimation program Interface for Windows, Version 3.02. Syracuse Research Corporation, Syracuse, NY, USA

acid and sodium AMPS® to be is approximately 100 gm/100 gm water and 150 gm/100 gm water, respectively.

2.2 Exposure Information for the AMPS® Category

AMPS® acid is manufactured and supplied as a white crystalline solid. The sodium and ammonium salts of AMPS® monomer are prepared as 50% aqueous solutions. AMPS® monomer is primarily used for the preparation of high molecular weight water-soluble polymers. AMPS® monomer can be polymerized in solution using conventional vinyl moiety polymerization. AMPS® is available as a crystalline solid or as an aqueous salt solution. AMPS® monomer is a highly reactive, hydrophilic sulfonic acid acrylic monomer capable of imparting a number of distinctive high-performance characteristics to a wide variety of ion-containing polymers and reaction products. The earliest patents using AMPS® monomer were filed for acrylic fiber manufacturing. Oil drilling, water treatment and coating/adhesive applications soon followed. Today, there are over 1,000 patents and publications involving manufacture and use of AMPS® monomer, representing a diversity of applications including cosmetics, medical technolo9gies, electrodeposition/plating, fuel and lubricant additives, flocculants, ion exchange resins, non-woven binders/adsorbents, photographic chemicals, paper, dispersants and textiles. AMPS® monomer can be polymerized in solution using conventional vinyl polymerization techniques. AMPS® monomer is low to hydrolyze in both its monomer and homopolymer configurations. As a result, AMPS® monomer imparts exceptional hydrolytic stability to resulting polymers.

2.3 Environmental Fate Data

Computer modeling techniques were used to evaluate the environmental fate and transport for members of the AMPS® category. If experimental data was not available, the physical-chemical properties of the AMPS® category members were estimated by using the EPIWIN model. The AMPS® acid was used as the prototype chemical for estimating the physical-chemical properties of this category. As stated above, the members of this category are characterized by a 2-acrylamido-2-methylpropanesulfonic parent anion, distinct only by the corresponding H^+, Na^+ or NH_4^+ counterion. For environmental fate estimations, the physical chemical characteristics of the acid is an appropriate read-across for the sodium and ammonium AMPS® salts included in this category. The physical-chemical properties (experimental and modeled data) of AMPS® acid are provided in Table 1A.

2.3.1 Fugacity Modeling

Fugacity is a thermodynamic term used to describe the behavior of a chemical in the environment. Fugacity-based multimedia modeling compares the relative distribution of chemicals between environmental compartments (i.e., air, soil, water, suspended sediment, sediment and biota). A widely used model for this approach is the Equilibrium Criterion Model[2] (EQC). There are 3 levels of the EQC model and EPA has recommended its use in the document titled, *"Determining the Adequacy of Existing*

[2] Mackay, et al. Evaluating the environmental fate of a variety of types of chemicals using the EQC Model. Environ. Toxicol. Chem. 15: 1627-1637

Data". In this document, EPA states that it accepts Level 1 fugacity modeling to estimate transport/distribution values. The EQC Level I model utilizes basic physical-chemical properties including molecular weight, vapor pressure, octanol-water partition coefficient and water solubility to calculate percent distribution within a standardized regional environment. The EQC Level II model also calculates the rates of transport and degradation within the environmental compartment. Application of the Level II model requires data on the rates of biodegradation, hydrolysis, photolysis and oxidation. EQC Level III evaluates the effects of discharge rates to air, water and soil and inter-media transport rates.

The EQC Level I model was used to estimate the relative distribution of AMPS® category chemicals among environmental compartments. The AMPS® acid was used as the representative chemical in the level 1 model. Results of the EQC Level 1 modeling shows that the AMPS® category members would partition almost exclusively into the water phase (Table 1B). Based on the high water solubility coupled with low soil adsorption and volatility, it is expected that chemicals in this group will partition preferentially into the water phase.

2.3.2 Hydrolysis

Hydrolysis is a chemical transformation process in which an organic molecule reacts with water, forming a new carbon-oxygen bond and cleaving a carbon-X bond in the original molecule, where X is the leaving group. Hydrolytic reactions depend on the susceptibility of the chemical to attack by a nucleophile such as a water molecule or hydroxide ion. Molecules that are susceptible to hydrolysis are those in which the electron distribution gives some charge separation, facilitating nucleophilic attack. The lack of a suitable leaving group renders compounds resistant to hydrolysis. Potentially hydrolyzable groups include esters, carbamates, epoxides, halomethanes and selected alkyl halides.

The potential for the AMPS® Category chemicals to undergo hydrolysis could not be evaluated using the EPIWIN model due to lack of hydrolyzable functional groups. However, the AMPS® monomer contains an amide functional group that could potentially hydrolyze into a carboxylic acid and an amine. In general, amides are much less hydrolytically reactive than esters and hydrolysis half-lives can range from hundreds to thousands of years in the aquatic environment. Shelf-live determinations indicated that aqueous solutions of sodium AMPS® at pH 9 resist hydrolysis for months under normal storage. All three members of the AMPS® category are structurally similar and can therefore be expected to exhibit similar hydrolysis characteristics. The hydrolytic stability likely results from the *gem*-dimethyl substitution adjacent to the amide group. Aqueous solutions of 2-acrylamido-2-methylpropanesulfonic anion will eventually hydrolyze to acrylic acid and dimethyltaurine. Based on the information available, the members of this category will not undergo significant hydrolysis and no additional testing is required.

2.3.3 Biodegradation

Biodegradation refers to transformation of chemicals by microorganisms where parts of the chemical are incorporated into cellular material or used as an energy source for the organisms, and the remainder is converted to simple inorganic molecules. The complete biodegradation of a substance to CO_2, H_2O and simple organic and inorganic end products is called mineralization. Biodegradability tests vary from those that only measure primary degradation (i.e., any biologically induced structural change in the parent compound) or ultimate degradation (i.e., complete conversion to inorganic compounds such as CO_2, H_2O; or methane if anaerobic microorganisms are involved). Primary degradation can be determined analytically by measuring dissolved organic carbon (DOC) for water-soluble chemicals by infrared absorbance, or by a chemical-specific method. Ultimate degradation (also called mineralization) is determined by measuring oxygen consumption or carbon dioxide evolution relative to the theoretical levels that can be achieved based on the elemental analysis.

Biodegradation can be measured using the OECD or U.S. EPA test guidelines. The biodegradation results for the compounds in this category are summarized in Table 2. A biodegradation test was conducted on AMPS® acid and sodium AMPS® using the semi-continuous activated sludge (SCAS) method (40CFR 795.3340). In the 44-day test, the biodegradation rate of both test materials was <10% based on dissolved organic carbon measurements. Based on the test results, both these compounds exhibited a very slow rate of biodegradability and are not readily biodegradable.

A Modified Sturm Test (OECD 301B) was conducted on ammonium AMPS®. In 28 days, 3.3% of the test material was converted to CO_2. Consequently, it was also assessed as exhibiting a very slow rate of biodegradability. Based on available data, all members of the category can be characterized as having a very slow rate of biodegradability and no additional tests are required.

2.3.4 Photodegradation

Photodegradation is the degradation of a chemical compound as a result of absorption of solar radiation. Environmental photo-reactions take place in the presence of sunlight only above 295nm in the near ultraviolet (UV) extending into the infrared region (750 nm) of the electromagnetic spectrum. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer. The presence of O_3 in the troposphere leads to the formation of OH radicals through the photolysis of O_3 at wavelengths ~ 290 to 350 nm. The OH radical is the key reactive species in the troposphere, reacting with all organic compounds apart from chlorofluorocarbons (CFCs) and other halogenated compounds which do not contain a hydrogen atom.

The tendency of the AMPS® acid to photodegrade was evaluated by using the modeling program EPIWIN which includes calculation of atmospheric oxidation potential (AOP). This computer simulation of photo-oxidation is recommended in the Agency's recently released structure activity review (SAR) guidance for HPV chemicals. This program

calculates chemical half-life based on an overall OH reaction rate constant, a 12-hour day, and a given OH concentration. The model results are provided below:

Overall OH Rate Constant = 16.1884 x 10^{-12} cm3/molecule-sec
Half life = 0.661 days (12-hr day; 1.5 x 10^{6} OH/cm^{3})

All three members of the AMPS® category are structurally similar and can therefore be expected to exhibit similar photodegradation characteristics. Based on the high water solubility and estimated low vapor pressure for members of this category, atmospheric oxidation is not likely to be a significant degradation pathway.

2.4 ECOTOXICOLOGY DATA

The purpose of the acute toxicity tests is to evaluate effects that occur rapidly as a result of short-term exposure to a chemical. Generally, acute effects are relatively severe, the most common one measured in aquatic organisms is lethality. A chemical is considered acutely toxic if by its direct action it causes mortality in 50% or more of the exposed population in a relatively short period of time, such as 96 hours to 14 days. Factors that may directly influence the toxicity of a chemical include solubility, vapor pressure, pH and lipophilicity. The chemicals in the AMPS® category are soluble in water, however, their low lipophilicity (as indicated by the low octanol water partition coefficient values) precludes significant bioconcentration in aquatic organisms. Available toxicity data summarized in Table 3 indicates that members of this category are not highly toxic to aquatic species.

2.4.1 Acute Fish Toxicity

Acute fish toxicity tests were conducted for both the AMPS® acid and sodium AMPS® in accordance with EPA-660/3-75-009 over a 96-hour exposure period. The median lethal concentration (LC50) was estimated to be 130 mg/L with the AMPS® acid and >1,000 with sodium AMPS®. Sublethal effects at 600 and 1,000-mg/L test solution was observed with the AMPS® acid whereas no effects were seen with sodium AMPS®.

An LC50 value of 1,400 mg/L was derived in a 96-hour fish toxicity test conducted with ammonium AMPS® in accordance with OECD 203. The test results suggest that the AMPS® acid is more toxic to fish than the corresponding salt. The acute fish toxicity data available for the AMPS® category chemicals is adequate and no further testing is required.

2.4.2 Acute Invertebrate Toxicity

Acute invertebrate toxicity tests were conducted for both the AMPS® acid and sodium AMPS® in accordance with EPA-660/3-75-009 over a 48-hour exposure period. The median effects concentration (EC50) was estimated to be 340 mg/L with the AMPS® acid and >1,000 with sodium AMPS®.

An EC50 value of 1,200 mg/L was derived in a 48-hour invertebrate toxicity test conducted with ammonium AMPS® in accordance with OECD 202. The test results suggest that the AMPS® acid is more toxic to the test organism than the corresponding

salt. The acute invertebrate toxicity data available for the AMPS® category chemicals is adequate and no further testing is required.

2.4.3 Algae Acute Toxicity

Acute algal toxicity test was conducted with ammonium AMPS® in accordance with OECD 201 over a 96-hour exposure period. The median effects concentration (EC50) was estimated to be >2,000 mg/L, the highest test concentration. Algal toxicity data is not available for the AMPS® acid or sodium AMPS®. Based on the structural similarity of the members of this category and relatively low toxicity, the data for ammonium AMPS® will be bridged to AMPS® acid and sodium AMPS®.

2.4.4 Summary of the Acute Aquatic Effects of Members of the AMPS® Category

Results of the acute toxicity tests show that the members of the AMPS® category are not significantly toxic to aquatic species. The LC50 and EC50 values in the fish and invertebrate toxicity tests respectively, were higher than 100 mg/L with all three members of this category. The AMPS® acid was slightly more toxic to the test organisms in both fish and invertebrate tests, compared to the sodium and ammonium AMPS®. In tests conducted with algae, the EC50 value for ammonium AMPS® was greater than 2,000 mg/L. Based on available data, it is apparent that the 2-acrylamido-2-methylpropanesulfonic parent anion present in all 3 members of this category, is not significantly toxic to aquatic organisms. The algae data available for ammonium AMPS® will be bridged to other members of this group and no additional testing is required.

2.5 HUMAN HEALTH TOXICOLOGICAL DATA

2.5.1 Acute Health Effects of the AMPS® Category

Acute health effects test results are summarized in Table 4.

2.5.1.1 Acute Oral Toxicity

Acute oral toxicity studies in rats are available for all three members of the AMPS® category. The acute oral toxicity studies presented were performed in accordance with OECD guidelines (OECD 401). The acute oral LD50 for AMPS® acid was 1830 mg/kg. As is reported in the robust summary for AMPS® acid, animal deaths were reported only at doses greater than 2000 mg/kg (range of doses 500-8000 mg/kg). Principle clinical findings following high oral doses of this strong acid were consistent with oral administration of a severe gastrointestinal irritant. In contrast, acute oral LD50s for the neutral salts of AMPS® monomer (i.e., sodium AMPS® and ammonium AMPS®) were greater than 5000 mg/kg. No unscheduled deaths were recorded after the administration of the AMPS® salts and no remarkable clinical observations were noted in the treated animals. The results of all three studies were deemed reliable without restriction according to the Klimisch criteria.

2.5.1.2 Acute Dermal Toxicity

The acute dermal toxicity studies were performed in accordance with OECD guidelines (OECD 402). These studies were performed in rabbits using ammonium AMPS®. The acute dermal LD50 was determined to be >2000 mg/kg. No unscheduled deaths were recorded in the study and no remarkable clinical observations or gross pathological findings suggestive of adverse systemic effects were noted. No preferential dermal toxicity was demonstrated. The results of this study were deemed reliable without restriction according to the Klimisch criteria.

2.5.1.3 Summary of the Acute Toxicological Effects of the AMPS® Category

Results of the acute toxicity studies indicate that the parent 2-acrylamido-2-methylpropanesulfonic anion does not exhibit direct systemic toxicity via the oral or dermal routes of administration. This is evidenced by the high acute oral LD50 seen with experiments using the neutral sodium and ammonium salts of AMPS® monomer, and the high LD50 observed following prolonged dermal application of ammonium AMPS®. The lower LD50 and adverse clinical findings associated with the oral administration of AMPS® acid are attributed to its strongly acidic properties resulting in severe local gastrointestinal reactions and the resulting secondary adverse physiological responses. Therefore, these acute toxicity results support the direct relationship between the physico-chemical properties and the acute toxicological findings for the members of the AMPS® category. Since all members of the group likely have an equally low level of toxicity under acute conditions, no additional testing is required.

2.5.2 Genetic Toxicology of the AMPS® Category

Genetic toxicity test results are summarized in Table 5.

2.5.2.1 Bacterial Gene Mutation Assay

In vitro reverse mutation assays using AMPS® acid were performed in six different strains of bacteria in accordance with international guidelines (combined OECD 471 and 472). Mutagenicity was studied with metabolic activation and in non-activated conditions. The conclusion of two separate investigations was that AMPS® acid was non-mutagenic under the conditions of the assay. The results of this study were deemed reliable without restriction according to the Klimisch criteria.

2.5.2.2 Mammalian Gene Mutation Assay

In vitro assays were performed in Chinese Hamster Ovary (CHO) cells to study the ability of AMPS® acid to induce mutations at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus. The methods used were consistent with OECD guideline 476. The test material was tested with, and without, metabolic activation. In two separate studies, no significant increase in the frequency of mutagenic events was detected in cells treated with AMPS® acid. The conclusion of these studies was that AMPS® acid was not mutagenic in mammalian systems. The results of one study was deemed reliable without restriction (Klimisch criteria) whereas the results of the other study were reliable with restrictions due to the lack of a metabolic activation test as recommended in OECD guidelines.

2.5.2.3 *In vitro* Chromosomal Aberration Assay

An *in vitro* assay was performed in Chinese hamster ovary (CHO) cells to study the ability of AMPS® acid to induce chromosomal aberrations. The method used was consistent with OECD guideline 473. The test material was tested with, and without, metabolic activation. The test material did not induce chromosomal aberrations in the non-activated assay, however, AMPS® acid exposed to hepatic microsomal activation produced non-dose-dependent increases in the frequency of chromosomal aberrations. The conclusion of this study was that metabolic activation of AMPS® acid may result in clastogenic activity. However, the absence of a dose-response effect, absence of a time-response effect, lack of reproducibility between experiments, coupled with the simultaneous occurrence of excessive cytotoxicity with the observed clastogenic events confounds the interpretation of the positive findings. The results of these studies were deemed reliable without restriction according to the Klimisch criteria. To further elucidate this potential, AMPS® acid was tested for the potential to produce chromosomal aberrations in an *in vivo* assay system.

2.5.2.4 *In vivo* Chromosomal Aberration Assay

In vivo assays were performed in two distinct assays in two species of mammals to study the ability of AMPS® monomer to induce chromosomal aberrations. A mouse micronucleus assay was performed according to OECD guideline 474. The clastogenic effect of intraperitoneal injection of AMPS® acid in male and female mice at doses ranging from 175-1750 mg/kg was measured at 24, 48 and 72 hours. These treatments did not produce statistically significant or dose-dependent increases in the frequency of chromosomal aberrations. In a further study, ammonium AMPS® was tested in a rat bone marrow cytogenetics test (OECD guideline 475). At doses ranging from 150-1500 mg/kg, oral administration of ammonium AMPS® to male and female rats did not produce statistically significant or dose-dependent increases in the frequency of chromosomal aberrations. The combined results of these tests indicate that AMPS® monomer is not clastogenic under *in vivo* assay conditions. The results of these studies were deemed reliable without restriction according to the Klimisch criteria.

2.5.2.5 Summary of the Genetic Toxicology of the AMPS® Category

Results of the genetic toxicity testing on AMPS® acid clearly show that the parent 2-acrylamido-2-methylpropanesulfonic anion is not a mutagen. Repeated negative findings in bacterial both and mammalian *in vitro* assay systems support this conclusion. The results of the single *in vitro* chromosomal aberration are inconclusive. Specifically, the clastogenic effects observed with the metabolic activation of AMPS® acid were not replicable and did not follow dose-responsive or time-responsive patterns. Furthermore, the clastogenic events were associated with excessive cytotoxic damage, and therefore may not represent direct genotoxic activity. Consequently, *in vivo* tests in rats and mice were performed to more reliably describe the cytogenetic toxicity of AMPS®. These rodent assays clearly demonstrate the absence of clastogenic potential resulting from the *in vivo* administration of AMPS® acid or ammonium AMPS® in mammalian systems. In conclusion, 2-acrylamido-2-methylpropanesulfonic anion is not considered to be a clastogen. Since AMPS® acid, sodium AMPS® and ammonium AMPS® are simple salts of the same parental anion, the lack of mutagenicity or *in vivo* clastogenic activity can be

extrapolated to the entire AMPS® category. Moreover, these combinations of genetic toxicity results further support the direct relationship between the physico-chemical properties and the toxicological findings for the entire membership of the AMPS® category. Since all members of the group likely have an equally low index of genetic toxicity, no additional testing is required.

2.5.3 Repeated Dose Health Effects of the AMPS® Category

Repeated dose health effect test results are summarized in Table 6.

2.5.3.1 Subchronic Oral Toxicity

A 28-day repeated dose oral toxicity test in rats was performed on ammonium AMPS®. This study was completed in accordance with OECD guideline 407. Five groups of male and female rats received doses of ammonium AMPS® ranging from 0 (water vehicle control) to 1000 mg/kg/day for 28 consecutive days, followed by a 14-day treatment-free recovery period. The were no deaths in the study, and the most remarkable clinical sign was gastrointestinal unrest manifested by lethargy, emaciation, diarrhea and reduced food consumption in a single male at the highest dose. Otherwise, there were no treatment-related untoward effects on clinical observations, body weight, food consumption, serum chemistry values, hematology values, gross pathological observations or histopathological findings. As a result, the laboratory study director assigned the no-observed-effect-level (NOEL) at 1000 mg/kg/day. The result of this study was deemed reliable without restriction according to the Klimisch criteria. The data in this study are reflective of a low subchronic toxicity index for ammonium AMPS®. In light of the strong physico-chemical and toxicological similarity between the members of the AMPS® category, this low index of subchronic toxicity can be applied to the category as a whole.

2.5.3.2 Reproductive/Developmental Toxicity

An assessment of the reproductive and developmental toxicity of ammonium AMPS® was performed using the method prescribed in OECD guideline 421. In this reproductive/developmental screen, male and female rats were dosed for two weeks prior to mating and, in the case of the females, through gestation, parturition and lactation day 4. Oral administration of the test material at doses of 100, 500 and 1000 mg/kg/day had no effect on F0 survival, growth, mating behavior, copulation, fertility, precoital intervals, gestation lengths, corpora lutea counts, implantation counts, mean live litter size, pre- or post-implantation loss, gross necropsy findings or organ weights (testes and epididymides). Histopathological examination of the testes, ovaries and epididymides from control and high-dose rats did not reveal any test material-related microscopic changes. No test material-related effects were observed in the F1 offspring with respect to survival, clinical observations, body weights or gross necropsy findings. In addition, there were no indications of test material-related developmental effects in the F1 pups at any dosage level tested. Based on the results of this study, the laboratory study director assigned both the reproductive NOEL and the developmental NOEL at 1000 mg/kg/day. The results of this study were deemed reliable without restriction according to the Klimisch criteria. This data indicated that ammonium AMPS® has a low index of reproductive or developmental toxicity. Based on the physico-chemical and toxicological

similarity of the members of the AMPS® category, this low index of reproductive/ developmental toxicity can be applied to all members of the AMPS® category.

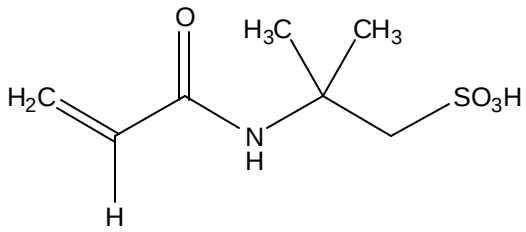
2.5.3.3 Summary of the Repeated Dose Toxicity data for the AMPS® Category

Results of the oral repeated dose toxicity testing on ammonium AMPS® clearly show that the parent 2-acrylamido-2-methylpropanesulfonic anion is not a cumulative or a reproductive/developmental toxicant. Repeated negative findings at high doses in both a subchronic repeated dose study and a reproductive/ developmental screening assay support this conclusion. Therefore, these repeated dose toxicity results support the direct relationship between the physico-chemical properties and the acute toxicological findings for the members of the AMPS® category. Since all members of the group likely have equally low index of subchronic or reproductive/developmental toxicity under repeated dose conditions, no additional testing of AMPS® acid or sodium AMPS® is required.

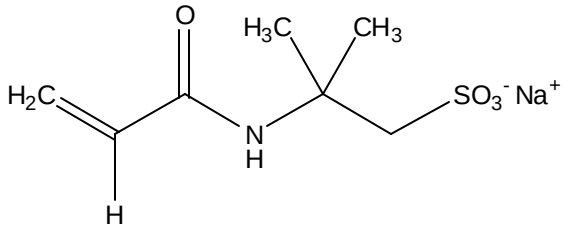
3.0 CONCLUSIONS

Physical-chemical, environmental fate, ecotoxicology and health-effects toxicity data (experimental or computer modeled) for the members of the AMPS® category has been evaluated for adequacy. The available data is of high quality and projects a profile of low overall toxicity. The consistency of the data between AMPS® acid, sodium AMPS® and ammonium AMPS® for these endpoints supports the AMPS® category approach. As a result, the use of conservative read-across within the category for existing data gaps will preclude any further testing.

Figure 1– Chemical Structures of the AMPS® Category



2-acrylamido-2-methylpropanesulfonic acid
CASRN 15214-89-8
AMPS® acid



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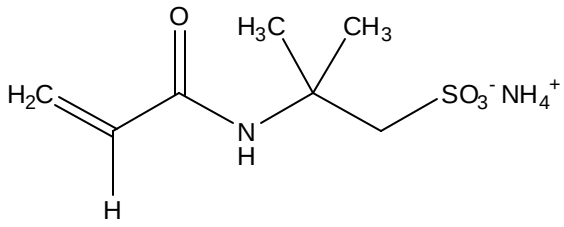
Sodium AMPS®



2-acrylamido-2-methylpropanesulfonic acid, ammonium salt
CASRN 58374-69-9
Ammonium AMPS®

Table 1A: Physical Properties and Environmental Fate Data for AMPS® acid

CAS #	Molecular Weight	Log K_{ow}	Water Solubility (mg/L)	Vapor Pressure (mm Hg)	Log K_{oc}	Log BCF	Melting Point (^oC)	Atmospheric Oxidation	
								OH^- Rate Constant (cm^3/molec-sec)	Half-life (hrs)
15214-89-8	207.25	-2.19	10^6	6.75E-09	1.0	0.5	160.78	16.3E-12	7.8

Table 1B: Predicted Environmental Distribution of AMPS® acid

CAS#	Air (%)	Water (%)	Soil (%)	Sediment (%)	Suspended Sediment (%)	Biota (%)	Fugacity (Pa)
15214-89-8	3.7E-09	100	5.7E-04	1.27E-05	3.97E-07	5.48E-03	4.5E-16

Table 2 - Evaluation of Environmental Fate Data for the AMPS® Category

CHEMICAL	BIODEGRADABILITY		HYDROLYSIS		PHOTODEGRADATION	
	AVAILABLE DATA	PROPOSED TESTING	AVAILABLE DATA	PROPOSED TESTING	AVAILABLE DATA	PROPOSED TESTING
AMPS® acid (CAS #15214-89-8)	Adequate data (<10% biodegraded after 44 days)	No testing needed- Adequate data	No data available	No testing needed- Bridging	EPIWIN Model Estimation (Half life = 0.661 days [12-hr day; 1.5 x 10^6 OH/cm^3])	No testing needed - Adequate data
Sodium AMPS® (CAS #5165-97-9)	Adequate data (<10% biodegraded after 44 days)	No testing needed- Adequate data	Yes (Resistant to hydrolysis)	No testing needed- Adequate data	No data available	No testing needed- Bridging
Ammonium AMPS® (CAS #58374-69-9)	Adequate data (<3.3% biodegraded after 28 days)	No testing needed- Adequate data	No data available	No testing needed- Bridging	No data available	No testing needed- Bridging

Table 3 – Evaluation of Aquatic Toxicity Data for the AMPS® Category

CHEMICAL (CAS #)	ACUTE TOXICITY TO FISH[1]			ACUTE TOXICITY TO INVERTEBRATES[2]			ACUTE TOXICITY TO ALGAE[3]		
	AVAILABLE DATA		PROPOSED TESTING	AVAILABLE DATA		PROPOSED TESTING	AVAILABLE DATA		PROPOSED TESTING
	96-hour LC_{50} (mg/L)	96-hour NOEC (mg/L)		48-hour EC_{50} (mg/L)	48-hour NOEC (mg/L)		96-hour EC_{50} (mg/L)	96-hour NOEC (mg/L)	
AMPS® acid (CAS #15214-89-8)	130	130 (LC0)	No testing needed – Adequate data	340	78 (LC0)	No testing needed – Adequate data	No data available	No data available	No testing needed - Bridging
Sodium AMPS® (CAS #5165-97-9)	>1,000	1,000 (LC0)	No testing needed – Adequate data	>1,000	1,000 (LC0)	No testing needed – Adequate data	No data available	No data available	No testing needed – Bridging
Ammonium AMPS® (CAS #58374-69-9)	1,400	640 (LC0)	No testing needed – Adequate data	1,200	640 (LC0)	No testing needed – Adequate data	>2,000	2,000 (LC0)	No testing needed – Adequate data

[1]Species is either Fathead minnow or Rainbow trout.
[2]Species is *Daphnia magna* unless otherwise noted.
[3]Species is freshwater algae *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*) unless otherwise noted.
[4]LC0 indicates concentration where no mortality or effects were seen.

Table 4 – Evaluation of Human Health Acute Toxicity Data for the AMPS® Category

	ACUTE ORAL TOXICITY		ACUTE DERMAL TOXICITY	
CHEMICAL (CAS #)	**AVAILABLE DATA**	**PROPOSED TESTING**	**AVAILABLE DATA**	**PROPOSED TESTING**
AMPS® acid (CAS #15214-89-8)	LD_{50} = 1830 mg/kg	No testing needed – Adequate data	No data available	No testing needed – Bridging
Sodium AMPS® (CAS #5165-97-9)	LD_{50} > 16,000 mg/kg	No testing needed – Adequate data	No data Available	No testing needed – Bridging
Ammonium AMPS® (CAS #58374-69-9)	LD_{50} > 5000 mg/kg	No testing needed – Adequate data	LD_{50} > 2000 mg/kg	No testing needed – Adequate data

Table 5 – Evaluation of Human Health Genetic Toxicity Data for the AMPS® Category

	Bacterial Mutagenicity		Mammalian Mutagenicity		*In vitro* chromosomal aberration		*In vivo* chromosomal aberration	
CHEMICAL (CAS #)	**AVAILABLE DATA**	**PROPOSED TESTING**	**AVAILABLE DATA**	**PROPOSED TESTING**	**AVAILABLE DATA**	**PROPOSED TESTING**	**AVAILABLE DATA**	**PROPOSED TESTING**
AMPS® acid (CAS #15214-89-8)	Non-mutagenic	No testing needed – Adequate data	Non-mutagenic	No testing needed – Adequate data	Suspicion of clastogenicity	No testing needed – Adequate data	Non-clastogenic	No testing needed – Adequate data
Sodium AMPS® (CAS #5165-97-9)	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging
Ammonium AMPS® (CAS #58374-69-9)	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging

Table 6– Evaluation of Human Health Repeated Dose Toxicity Data for the AMPS® Category

	28-DAY REPEATED DOSE TOXICITY		REPRODUCTIVE/DEVELOPMENTAL TOXICITY	
CHEMICAL (CAS #)	**AVAILABLE DATA**	**PROPOSED TESTING**	**AVAILABLE DATA**	**PROPOSED TESTING**
AMPS® acid (CAS #15214-89-8)	No data Available	No testing needed – Bridging	No data Available	No testing needed – Bridging
Sodium AMPS® (CAS #5165-97-9)	No data Available	No testing needed – Bridging	No data Available	No testing needed – Bridging
Ammonium AMPS® (CAS #58374-69-9)	NOEL = 1000 mg/kg/day	No testing needed – Adequate data	F0 NOEL = 1000 mg/kg/day F1 NOEL = 1000 mg/kg/day	No testing needed – Adequate data

Table 7 – Test Plan for AMPS® Category

	AMPS® acid	Sodium **AMPS®**	Ammonium **AMPS®**
Physico-Chemical			
Melting Point	**X^{m}**	**X^{m}**	**X**
Boiling Point	NA	NA	NA
Vapor Pressure	**X^{m}**	**X^{m}**	X
Partition Coefficient	**X^{m}**	**X^{m}**	X
Water Solubility	**X^{m}**	**X^{m}**	X
Environmental Fate			
Photodegradation	**X^{m}**	x	x
Hydrolysis	x	x	**X**
Fugacity	**X^{m}**	x	x
Biodegradation	**X**	**X**	**X**
Ecological Toxicity			
Acute Fish	**X**	**X**	**X**
Acute Algae	x	x	**X**
Acute Daphnia	**X**	**X**	**X**
Mammalian Toxicity			
Acute Health (oral &/or dermal)	**X**	**X**	**X**
Bacterial Mutagenicity	**X**	x	x
Mammalian Mutagenicity	**X**	x	x
In vitro Cytogenetics	**X**	x	x
In vivo Cytogenetics	X	x	**X**
Repeated Dose Toxicity	x	x	**X**
Repro/Developmental Toxicity	x	x	**X**

Where:

X – Actual test data
x – Read-across from other members of the AMPS® category
X^{m} – Modeling data
NA – Endpoint not applicable for chemical